Bioo Scientific®

FOR REFERENCE PURPOSES

This manual is for Reference Purposes Only. DO NOT use this protocol to run your assays. Periodically, optimizations and revisions are made to the kit and protocol, so it is important to always use the protocol included with the kit.

NEXTflex™ Rapid Directional RNA-Seq Kit

(Illumina Compatible)

Catalog #5138-07 (8 reactions)

innovación tecnológica para laboratorio Rafer

 V14.09

This product is for research use only.

NEXTflex™ Rapid Directional RNA-Seq Kit - 5138-07

Product Overview

The NEXTflex™ Rapid Directional RNA-Seq Kit is designed to prepare directional, strand specific RNA libraries for sequencing using Illumina® sequencers. Bioo Scientific's NEXTflex™ Rapid Directional RNA-Seq Kit streamlines the sample prep procedure required for single, paired-end and multiplexed sequencing of RNA. NEXTflex Rapid Directional RNA-Seq simplifies workflow by using master mixed reagents and magnetic bead based cleanup, reducing pipetting and eliminating time consuming steps in library preparation. In addition, the availability of up to 96 unique adapter barcodes and gel-free size selection allows for high-throughput, multiplexed sequencing. The procedure is ideal for insert sizes of >150 bp.

This kit can be used to generate 8 RNA libraries. There are six steps involved in preparing RNAs for sequencing: RNA extraction and enrichment, fragmentation, first strand and second strand synthesis, adenylation, adapter ligation, and PCR amplification. Directionality is retained by adding dUTP during the second strand synthesis step and subsequent cleavage of the uridine-containing strand using Uracil DNA Glycosylase. The strand that's sequenced is the cDNA strand. This kit contains the necessary reagents to process the user's purified RNA sample through preparation and amplification for loading onto flow cells for sequencing.

Contents, Storage and Shelf Life

The NEXTflex™ Rapid Directional RNA-Seq Kit contains enough material to prepare 8 RNA samples for Illumina® compatible sequencing. The shelf life of all reagents is 12 months when stored properly. All components can safely be stored at -20°C.

Kit Contents	Amount
BROWN CAP	
NEXTflex™ RNA Fragmentation Buffer	40 µL
RED CAP	
NEXTflex™ First Strand Synthesis Primer	8 µL
NEXTflex™ Directional First Strand Synthesis Buffer Mix	32 µL
NEXTflex™ Rapid Reverse Transcriptase	8 µL
BLUE CAP	
NEXTflex™ Directional Second Strand Synthesis Mix	200 µL
PINK CAP	
NEXTflex™ Adenylation Mix	36 µL
YELLOW CAP	
NEXTflex™ Ligation Mix	220 µL
NEXTflex™ RNA-Seq Barcode Adapter 1 (0.6 µM)	16 µL

GREEN CAP	
NEXTflex™ Uracil DNA Glycosylase	8 µL
NEXTflex™ Primer Mix (12.5 µM)	16 µL
NEXTflex™ PCR Master Mix	96 µL

GRAY CAP	
Nuclease-free Water	1.5 mL

WHITE CAP	
Resuspension Buffer	(2) 1 mL

Required Materials not Provided

- Total RNA Input: 10 ng – 1 µg total RNA for enrichment by NEXTflex™ Poly(A) Beads (Cat. # 512979, 512980, 512981). NEXTflex™ Poly(A) Beads are available separately.
- mRNA Input: ~1 ng - 100 ng isolated mRNA.
- rRNA-depleted RNA Input: ~1ng - 100 ng. Bioo Scientific recommends Ribo-Zero™ (Epicentre) or RiboMinus™ (Life Technologies) for rRNA depletion.
- 100% Ethanol (stored at room temperature)
- 80% Ethanol (stored at room temperature)
- 2, 10, 20, 200 and 1000 µL pipettes
- RNase-free pipette tips
- Nuclease-free 1.5 mL microcentrifuge tubes
- Thin wall nuclease-free 0.5 mL microcentrifuge tubes
- 96 well PCR Plate Non-skirted (Phenix Research, Cat # MPS-499) or similar
- Adhesive PCR Plate Seal (BioRad, Cat # MSB1001)
- Agencourt AMPure XP 60 mL (Beckman Coulter Genomics, Cat # A63881)
- Magnetic Stand -96 (Ambion, Cat # AM10027) or similar for post PCR cleanup
- Microcentrifuge
- Thermocycler
- Heat block
- Vortex

Optional Materials not Provided

- NEXTflex™ RNA-Seq Barcodes – 6 / 12 / 24 / 48 (Cat # 512911, 512912, 512913, 512914)
- NEXTflex-96™ RNA-Seq Barcodes (Cat # 512915, 512916)

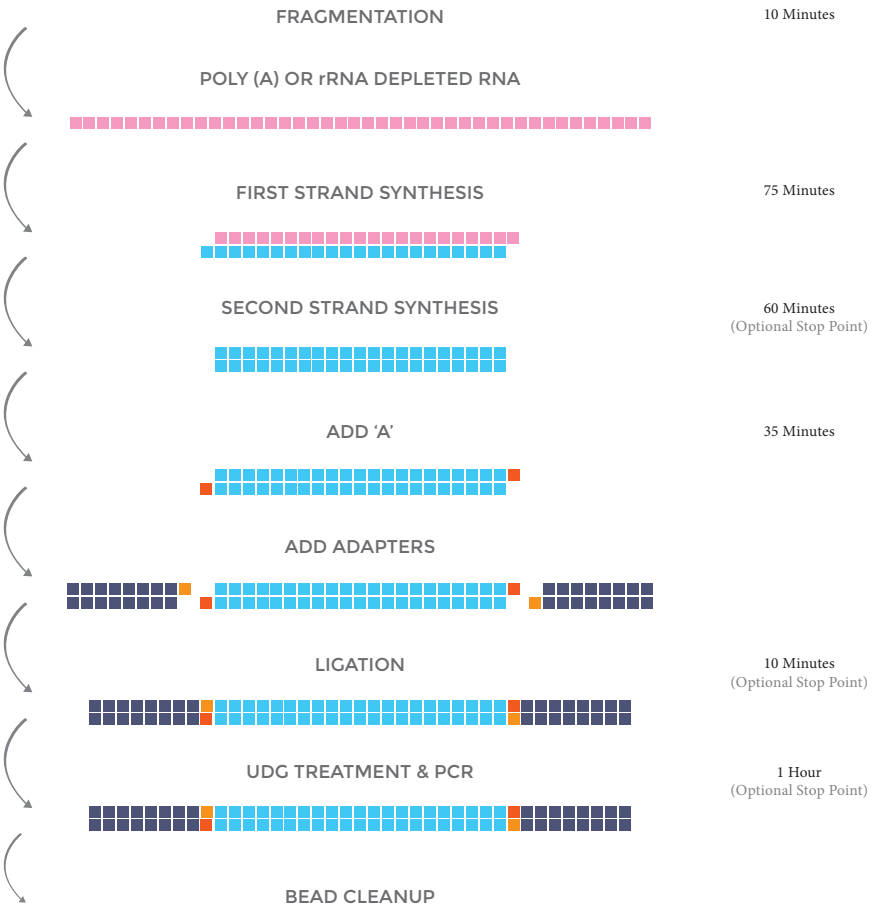
Warnings and Precautions

Bioo Scientific strongly recommends that you read the following warnings and precautions. Periodically, optimizations and revisions are made to the components and manual. Therefore, it is important to follow the protocol included with the kit. If you need further assistance, you may contact your local distributor or Bioo Scientific at nextgen@biooscientific.com.

- Do not use the kit past the expiration date.
- The NEXTflex™ Directional First Strand Synthesis Buffer Mix is yellow in color.
- DTT in buffers may precipitate after freezing. If precipitate is seen, vortex buffer for 1-2 minutes or until the precipitate is in solution. The performance of the buffer is not affected once precipitate is in solution.
- Ensure pipettes are properly calibrated as library preparations are highly sensitive to pipetting error.
- Do not heat the RNA-Seq Adapters above room temperature.
- This kit contains a single Barcoded Adapter. To enable multiplexing, please use the appropriate combination of NEXTflex™ RNA-Seq Barcodes during the Adapter Ligation step.
- Try to maintain a laboratory temperature of 20°–25°C (68°–77°F).
- RNA sample quality may vary between preparations. High quality RNA should have a 28S band that is twice as intense as the 18S band of ribosomal RNA.
- Vortex and micro-centrifuge each component prior to use, to ensure material has not lodged in the cap or the side of the tube.

NEXTflex™ Rapid Directional RNA-Seq Sample Preparation Flow Chart

Figure 1: Sample flow chart with approximate times necessary for each step.



Starting Material

The NEXTflex™ Rapid Directional RNA-Seq™ Kit has been optimized and validated using poly(A) enriched or rRNA depleted RNA (~1 ng - 100 ng). Only 10 ng - 1 µg of total RNA are required if NEXTflex™ Poly(A) beads are used to enrich for mRNA*. Best results are obtained when using 50 ng or more of high quality total RNA.

Bioo Scientific recommends examining total RNA integrity using an Agilent Bioanalyzer. High quality total RNA preparations should have an RNA Integrity Number (RIN) greater than or equal to 8. Alternatively, total RNA may be run on a 1 - 2% agarose gel and integrity examined by staining with ethidium bromide. High quality RNA should have a 28S band that is twice as intense as the 18S band of ribosomal RNA. Lower amounts of starting material result in higher duplication rates and other changes in sequencing data quality.

*Low RNA inputs may reduce library complexity. Read our application note about constructing high quality RNA-Seq libraries from limited amounts of total RNA. Request a PDF copy by emailing BiooNGS@BiooScientific.com.

Revision History

Version	Date	Description
V13.07	July 2013	Initial Product Launch
V14.09	September 2014	The starting material necessary has been reduced to 10 ng total RNA when poly(A) enriched over NEXTflex™ Poly(A) Beads (Cat. # 512979, 512980, 512981).

STEP A: RNA Fragmentation

Materials

Bioo Scientific Supplied

BROWN CAP - NEXTflex™ RNA Fragmentation Buffer

GRAY CAP - Nuclease-free Water

User Supplied

mRNA enriched from 10 ng – 1 µg total RNA by NEXTflex™ Poly(A) Beads, or ~1 ng - 100 ng mRNA/rRNA-depleted RNA in up to 14 µL Nuclease-free Water or Elution Buffer
Nuclease-free microcentrifuge tube or plate
Thermocycler or heatblock set to 95°C
Ice

1. For each reaction combine the following in a nuclease-free microcentrifuge tube or plate:

14 µL	RNA (in Nuclease-free Water or Elution Buffer)
5 µL	NEXTflex™ RNA Fragmentation buffer
19 µL	TOTAL

2. Mix thoroughly by pipetting.
3. Heat for 10 minutes at 95°C, immediately place on ice.
4. Proceed to Step B: First Strand Synthesis.

STEP B: First Strand Synthesis

Materials

Bioo Scientific Supplied

RED CAP - NEXTflex™ First Strand Synthesis Primer, NEXTflex™ Directional First Strand Synthesis Buffer Mix, NEXTflex™ Rapid Reverse Transcriptase

User Supplied

Fragmented RNA (from Step A)
Thermocycler
Ice

1. For each reaction, add 1 µL NEXTflex™ First Strand Synthesis Primer to the fragmented RNA (from Step A).
2. Incubate at 65°C for 5 minutes, and immediately place on ice.
3. For each reaction, combine the following in a nuclease-free microcentrifuge tube or plate:

20 µL	Fragmented RNA + NEXTflex™ First Strand Synthesis Primer
4 µL	NEXTflex™ Directional First Strand Synthesis Buffer Mix
1 µL	NEXTflex™ Rapid Reverse Transcriptase
25 µL	TOTAL

4. Mix thoroughly by pipetting.
5. Incubate at:

10 min	25°C
50 min	50°C
10 min	70°C

6. Proceed to Step C: Second Strand Synthesis.

STEP C: Second Strand Synthesis

Materials

Bioo Scientific Supplied

BLUE CAP - NEXTflex™ Directional Second Strand Synthesis Mix

User Supplied

First Strand Synthesis product (from Step B)
Thermocycler
Ice

1. For each reaction combine the following in a nuclease-free microcentrifuge tube or plate:

25 µL	First Strand Synthesis product (from Step B)
25 µL	NEXTflex™ Directional Second Strand Synthesis Mix (contains dUTP)
50 µL	TOTAL

2. Mix thoroughly by pipetting
3. Incubate for 60 minutes at 16°C.
4. Proceed to Step D: Bead Cleanup.

STEP D: Bead Cleanup

Materials

Bioo Scientific Supplied
WHITE CAP - Resuspension Buffer

User Supplied
Second Strand Synthesis DNA (from Step C)
Adhesive PCR Plate Seal
Agencourt AMPure XP Magnetic Beads (room temperature)
80% Ethanol, freshly prepared (room temperature)
Magnetic Stand

1. Add 90 µL of well mixed AMPure XP Beads to each sample. Mix thoroughly by pipetting.
2. Incubate the plate for 5 minutes at room temperature.
3. Place the plate on the magnetic stand for 5 minutes at room temperature or until the supernatant appears completely clear.
4. Remove and discard all of the supernatant from the plate taking care not to disturb the beads.
5. With plate on stand, add 200 µL of freshly prepared 80% ethanol to each well without disturbing the beads and incubate the plate for at least 30 seconds at room temperature. Carefully, remove and discard the supernatant.
6. Repeat step 5, for a total of two ethanol washes. Ensure the ethanol has been removed.
7. Remove the plate from the magnetic stand and let dry at room temperature for 2 minutes.
8. Resuspend dried beads in 17 µL of Resuspension Buffer. Mix thoroughly by pipetting. Ensure that the beads are completely rehydrated and resuspended.
9. Incubate resuspended beads at room temperature for 2 minutes.
10. Place the plate on the magnetic stand for 5 minutes at room temperature or until the supernatant appears completely clear.
11. Transfer 16 µL of the clear supernatant to a fresh well for the next step.
12. The procedure may be stopped at this point and the reactions stored at -20°C.

STEP E: Adenylation

Materials

Bioo Scientific Supplied

PINK CAP - NEXTflex™ Adenylation Mix

User Supplied

Purified Second Strand Synthesis DNA (from Step D)
Thermocycler
Ice

1. For each sample, combine the following reagents on ice in a nuclease-free 96 well PCR Plate:

16 µL	Second strand synthesis DNA (from Step D)
4.5 µL	NEXTflex™ Adenylation Mix
20.5 µL	TOTAL

2. Mix thoroughly by pipetting.
3. Incubate at:

30 min	37°C
5 min	70°C

4. Proceed to Step F: Adapter Ligation.

STEP F: Adapter Ligation

Materials

Bioo Scientific Supplied

YELLOW CAP - NEXTflex™ Ligation Mix (remove right before use and store immediately after use at -20°C), NEXTflex™ RNA-Seq Barcode Adapter 1 or NEXTflex™ RNA-Seq Barcodes – 6 / 12 / 24 / 48 / 96 (Cat # 512911, 512912, 512913, 512914, 512915, 512916)

User Supplied

3' Adenylated DNA (from Step E)
Thermocycler

1. For each sample, combine the following reagents on ice in a nuclease-free 96 well PCR Plate:

20.5 µL	3' Adenylated DNA (from Step E)
27.5 µL	NEXTflex™ Ligation Mix
2.0 µL	NEXTflex™ RNA-Seq Barcode Adapter 1 or RNA-Seq Barcode
50 µL	TOTAL

2. Mix thoroughly by pipetting.
3. Incubate on a thermocycler for 10 minutes at 30°C.
4. Proceed to Step G: Bead Cleanup.

STEP G: Bead Cleanup

Materials

Bioo Scientific Supplied
WHITE CAP - Resuspension Buffer

User Supplied
Adapter Ligated DNA (from Step F)
Adhesive PCR Plate Seal
Agencourt AMPure XP Magnetic Beads (room temperature)
80% Ethanol, freshly prepared (room temperature)
Magnetic Stand

1. Add 50 µL of well mixed AMPure XP Beads to each well containing sample. Mix thoroughly by pipetting.
2. Incubate the plate for 5 minutes at room temperature.
3. Place the plate on the magnetic stand for 5 minutes at room temperature or until the supernatant appears completely clear.
4. Remove and discard all of the supernatant from the plate taking care not to disturb the beads.
5. With plate on stand, add 200 µL of freshly prepared 80% ethanol to each well without disturbing the beads and incubate the plate for at least 30 seconds at room temperature. Carefully, remove and discard the supernatant.
6. Repeat step 5, for a total of two ethanol washes. Ensure the ethanol has been removed.
7. Remove the plate from the magnetic stand and let dry at room temperature for 2 minutes or until bead pellet is dry.
8. Resuspend dried beads in 51 µL of Resuspension Buffer. Mix thoroughly by pipetting. Ensure that the beads are completely rehydrated and resuspended.
9. Incubate resuspended beads at room temperature for 2 minutes.
10. Place the plate on the magnetic stand for 5 minutes at room temperature or until the supernatant appears completely clear.
11. Transfer 50 µL of the clear supernatant to a fresh well.
12. Add 50 µL of well mixed AMPure XP Beads to each well containing sample. Mix thoroughly by pipetting.
13. Incubate the plate for 5 minutes at room temperature.
14. Place the plate on the magnetic stand for 5 minutes at room temperature or until the supernatant appears completely clear.
15. Remove and discard all of the supernatant from the plate taking care not to disturb the beads.

16. With plate on stand, add 200 µL of freshly prepared 80% ethanol to each well without disturbing the beads and incubate the plate for at least 30 seconds at room temperature. Carefully, remove and discard the supernatant.
17. Repeat step 16, for a total of two ethanol washes. Ensure the ethanol has been removed.
18. Remove the plate from the magnetic stand and let dry at room temperature for 2 minutes or until bead pellet is dry.
19. Resuspend dried beads in 36 µL of Resuspension Buffer. Mix thoroughly by pipetting. Ensure that the beads are completely rehydrated and resuspended.
20. Incubate resuspended beads at room temperature for 2 minutes.
21. Place the plate on the magnetic stand for 5 minutes at room temperature or until the supernatant appears completely clear.
22. Transfer 35 µL of the clear supernatant to a fresh well for the next step.
23. The procedure may be stopped at this point and the reactions stored at -20°C.

STEP H: PCR Amplification

Materials

Bioo Scientific Supplied

GREEN CAP - NEXTflex™ Uracil DNA Glycosylase, NEXTflex™ Primer Mix, NEXTflex™ PCR Master Mix

User Supplied

Purified Adapter Ligated DNA (from Step G)
Thermocycler
96 Well PCR Plate

1. For each sample, combine the following reagents on ice in the 96 well PCR plate:

35 µL	Adapter Ligated DNA
1 µL	NEXTflex™ Uracil DNA Glycosylase
12 µL	NEXTflex™ PCR Master Mix
2 µL	NEXTflex™ Primer Mix
50 µL	TOTAL

2. Mix thoroughly by pipetting.
3. PCR cycles:

30 min	37°C	
2 min	98°C	
30 sec	98°C	Repeat 15 cycles*
30 sec	65°C	
60 sec	72°C	
4 min	72°C	

*PCR cycles will vary depending on the amount of starting material and quality of your sample. Further optimization may be necessary. Always use the least number of cycles possible.

4. Proceed to Step I: Bead Cleanup.

STEP I: Bead Cleanup

Materials

Bioo Scientific Supplied
WHITE CAP - Resuspension Buffer

User Supplied
PCR Amplified DNA (from Step H)
Adhesive PCR Plate Seal
Agencourt AMPure XP Magnetic Beads (room temperature)
80% Ethanol, freshly prepared (room temperature)
Magnetic Stand

1. Add 40 µL of well mixed AMPure XP Beads to each well containing sample. Mix thoroughly by pipetting.
2. Incubate the plate for 5 minutes at room temperature.
3. Place the plate on the magnetic stand for 5 minutes at room temperature or until the supernatant appears completely clear.
4. Remove and discard all of the supernatant from the plate taking care not to disturb the beads.
5. With plate on stand, add 200 µL of freshly prepared 80% ethanol to each well without disturbing the beads and incubate the plate for at least 30 seconds at room temperature. Carefully, remove and discard the supernatant.
6. Repeat step 5, for a total of two ethanol washes. Ensure the ethanol has been removed.
7. Remove the plate from the magnetic stand and let dry at room temperature for 2 minutes or until bead pellet is dry.
8. Resuspend dried beads in 51 µL of Resuspension Buffer. Mix thoroughly by pipetting. Ensure that the beads are completely rehydrated and resuspended.
9. Incubate resuspended beads at room temperature for 2 minutes.
10. Place the plate on the magnetic stand for 5 minutes at room temperature or until the supernatant appears completely clear.
11. Transfer 50 µL of the clear supernatant to a fresh well.
12. Add 40 µL of well mixed AMPure XP Beads to each well containing sample. Mix thoroughly by pipetting.
13. Incubate the plate for 5 minutes at room temperature.
14. Place the plate on the magnetic stand for 5 minutes at room temperature or until the supernatant appears completely clear.
15. Remove and discard all of the supernatant from the plate taking care not to disturb the beads.

16. With plate on stand, add 200 µL of freshly prepared 80% ethanol to each well without disturbing the beads and incubate the plate for at least 30 seconds at room temperature. Carefully, remove and discard the supernatant.

17. Repeat step 16, for a total of two ethanol washes. Ensure the ethanol has been removed.

18. Remove the plate from the magnetic stand and let dry at room temperature for 2 minutes or until bead pellet is dry.

19. Resuspend dried beads in 32 µL of Resuspension Buffer. Mix thoroughly by pipetting. Ensure that the beads are completely rehydrated and resuspended.

20. Incubate resuspended beads at room temperature for 2 minutes.

21. Place the plate on the magnetic stand for 5 minutes at room temperature or until the supernatant appears completely clear.

22. Transfer 30 µL of the clear supernatant to a fresh well.

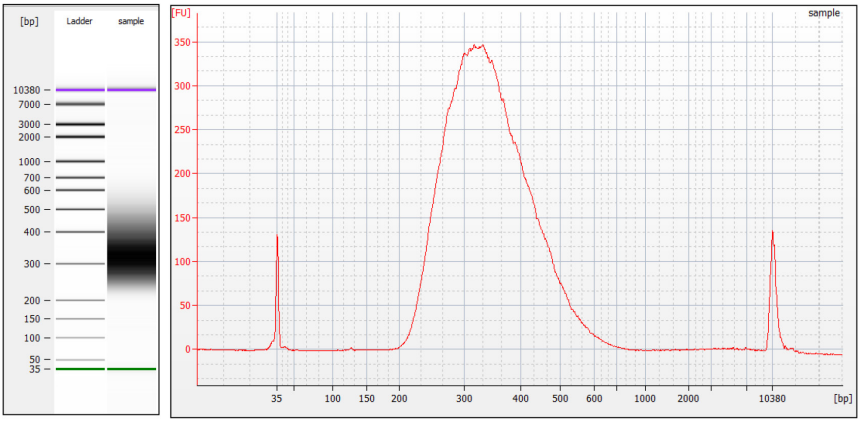
23. We recommend quantifying your library with a fluorometer and checking the size using an Agilent Bioanalyzer. If on the Bioanalyzer trace there are two bands, one of expected size and one of higher molecular weight, a portion of your adapter ligated inserts have annealed to each other forming a bubble product. This occurs due to the long adapter length and is more prevalent when there are too many PCR cycles. This type of double band will not affect your sequencing results as the double stranded product will be denatured prior to cluster generation. As an extra verification step, a portion of your product can be denatured manually by heating the sample to 95°C for 5 minutes and then placing it on ice. The denatured product should appear as a single band on a Bioanalyzer RNA Pico 6000 Chip Kit.

24. qPCR is recommended to quantitate DNA library templates for optimal cluster density. This can be performed using any qPCR quantification kit with the NEXTflex™ Primer Mix.

25. Non-multiplexed libraries can be normalized using Tris-HCl (10 mM), pH 8.5 with 0.1% Tween 20. For multiplexed libraries, transfer 10 µL of each normalized library for pooling in the well of a new 96 Well PCR Plate. Mix thoroughly by pipetting.

26. The library is now ready for cluster generation per the standard Illumina protocol. Proceed to cluster generation or store at -20°C.

Figure 2 : Example of mRNA library size distribution. 1 µL of the library was run on an Agilent High Sensitivity DNA chip to verify size. Using a Qubit® 2.0 Fluorometer & Qubit® dsDNA HS Assay Kit, the concentration of the library was determined to be > 10 nM.



Oligonucleotide Sequences

NEXTflex™	Sequence
PCR Primer 1	5'AATGATACGGCGACCACCGAGATCTACAC
PCR Primer 2	5'CAAGCAGAAGACGGCATACGAGAT
RNA- Seq Barcode Adapter 1	5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT 5'GATCGGAAGAGCACACGTCTGAACTCCAGTCACCGATGTATCTCGTATGCCGTCTTCTGCTTG

Illumina Compatible RNA NGS Kits and Adapters

Catalog #	Product
5138-01	NEXTflex™ Rapid RNA-Seq Kit (8 reactions)
5138-02	NEXTflex™ Rapid RNA-Seq Kit (48 reactions)
5138-07	NEXTflex™ Rapid Directional RNA-Seq Kit (8 reactions)
5138-08	NEXTflex™ Rapid Directional RNA-Seq Kit (48 reactions)
512911	NEXTflex™ RNA-Seq Barcodes –6
512912	NEXTflex™ RNA-Seq Barcodes – 12
512913	NEXTflex™ RNA-Seq Barcodes – 24
512914	NEXTflex™ RNA-Seq Barcodes – 48
512916	NEXTflex-96™ RNA-Seq Barcodes
5130-01	NEXTflex™ qRNA-Seq™ Kit 4 barcodes (8 reactions)
5130-02	NEXTflex™ qRNA-Seq™ Kit 24 barcodes - Set A (48 reactions)
5130-03	NEXTflex™ qRNA-Seq™ Kit 24 barcodes - Set B (48 reactions)
5130-04	NEXTflex™ qRNA-Seq™ Kit 24 barcodes - Set C (48 reactions)
5130-05	NEXTflex™ qRNA-Seq™ Kit 24 barcodes - Set D (48 reactions)
5130-01D	NEXTflex™ Rapid Directional qRNA-Seq™ Kit 4 barcodes (8 reactions)
5130-02D	NEXTflex™ Rapid Directional qRNA-Seq™ Kit 24 barcodes - Set A (48 reactions)
5130-03D	NEXTflex™ Rapid Directional qRNA-Seq™ Kit 24 barcodes - Set B (48 reactions)
5130-04D	NEXTflex™ Rapid Directional qRNA-Seq™ Kit 24 barcodes - Set C (48 reactions)
5130-05D	NEXTflex™ Rapid Directional qRNA-Seq™ Kit 24 barcodes - Set D (48 reactions)
5132-01	NEXTflex™ Small RNA Sequencing Kit (24 reactions)
5132-02	NEXTflex™ Small RNA Sequencing Kit (48 reactions)
5132-03	NEXTflex™ Small RNA Sequencing Kit v2 (24 reactions)
5132-04	NEXTflex™ Small RNA Sequencing Kit v2 (48 reactions)
513305	NEXTlfex™ Small RNA Barcode Primers -12 (Set A)
513306	NEXTlfex™ Small RNA Barcode Primers -12 (Set B)
513307	NEXTlfex™ Small RNA Barcode Primers -12 (Set C)
513308	NEXTlfex™ Small RNA Barcode Primers -12 (Set D)
512979	NEXTflex™ Poly(A) Beads (8 reactions)
512980	NEXTflex™ Poly(A) Beads (48 reactions)
512981	NEXTflex™ Poly(A) Beads (100 reactions)

Illumina Compatible DNA NGS Kits and Adapters

Catalog #	Product
4201-01	NEXTflex™ 16S V4 Amplicon-Seq Kit – 4
4201-02	NEXTflex™ 16S V4 Amplicon-Seq kit – 12
4201-03	NEXTflex™ 16S V4 Amplicon-Seq kit – 24
4201-04	NEXTflex™ 16S V4 Amplicon-Seq kit – 48
4201-05	NEXTflex™ 16S V4 Amplicon-Seq kit – 96
4201-06	NEXTflex™ 16S V4 Amplicon-Seq kit – 192
4201-07	NEXTflex™ 16S V4 Amplicon-Seq kit – 288
4202-01	NEXTlfex™ 16S V1-V3 Amplicon-Seq Kit - 4
4202-02	NEXTlfex™ 16S V1-V3 Amplicon-Seq Kit - 12
4202-03	NEXTlfex™ 16S V1-V3 Amplicon-Seq Kit - 48
4202-04	NEXTlfex™ 16S V1-V3 Amplicon-Seq Kit - 1-96
4202-05	NEXTlfex™ 16S V1-V3 Amplicon-Seq Kit - 97-192
4202-06	NEXTlfex™ 16S V1-V3 Amplicon-Seq Kit - 193-288
4202-07	NEXTlfex™ 16S V1-V3 Amplicon-Seq Kit - 289-384
5140-01	NEXTflex™ DNA Sequencing Kit (8 reactions)
5140-02	NEXTflex™ DNA Sequencing Kit (48 reactions)
5144-01	NEXTflex™ Rapid DNA-Seq Kit (8 reactions)
5144-02	NEXTflex™ Rapid DNA-Seq Kit (48 reactions)
5150-01	NEXTflex™ Cell Free DNA-Seq Kit (8 reactions)
5150-02	NEXTflex™ Cell Free DNA-Seq Kit (48 reactions)
514101	NEXTflex™ DNA Barcodes – 6
514102	NEXTflex™ DNA Barcodes – 12
514103	NEXTflex™ DNA Barcodes – 24
514104	NEXTflex™ DNA Barcodes – 48
514105	NEXTflex-96™ DNA Barcodes (Plate Format)
514106	NEXTflex-96™ DNA Barcodes (Tube Format)
514160	NEXTflex™ Dual-Indexed DNA Barcodes (1-96)
514161	NEXTflex™ Dual-Indexed DNA Barcodes (97-192)
5119-01	NEXTflex™ Bisulfite-Seq kit (8 reactions)
5119-02	NEXTflex™ Bisulfite-Seq kit (48 reactions)
511911	NEXTflex™ Bisulfite-Seq Barcodes – 6
511912	NEXTflex™ Bisulfite-Seq Barcodes – 12
511913	NEXTflex™ Bisulfite-Seq Barcodes - 24
5118-01	NEXTflex™ Methyl-Seq 1 Kit (8 reactions)
5118-02	NEXTflex™ Methyl-Seq 1 Kit (48 reactions)

511921	NEXTflex™ Msp 1 (8 reactions)
511922	NEXTflex™ Msp 1 (48 reactions)

5143-01	NEXTflex™ ChIP-Seq Kit (8 reactions)
5143-02	NEXTflex™ ChIP-Seq Kit (48 reactions)
514120	NEXTflex™ ChIP-Seq Barcodes – 6
514121	NEXTflex™ ChIP-Seq Barcodes – 12
514122	NEXTflex™ ChIP-Seq Barcodes – 24
514123	NEXTflex™ ChIP-Seq Barcodes – 48
514124	NEXTflex-96™ ChIP-Seq Barcodes

5140-51	NEXTflex™ Pre-Capture Combo Kit (6 barcodes)
5140-52	NEXTflex™ Pre-Capture Combo Kit (12 barcodes)
5140-53	NEXTflex™ Pre-Capture Combo Kit (24 barcodes)
5140-56	NEXTflex™ Pre-Capture Combo Kit (48 barcodes)
5140-54	NEXTflex™ Pre-Capture Combo Kit (96 barcodes)
514131	NEXTflex™ DNA Barcode Blockers - 6 for SeqCap
514132	NEXTflex™ DNA Barcode Blockers - 12 for SeqCap
514133	NEXTflex™ DNA Barcode Blockers - 24 for SeqCap
514136	NEXTflex™ DNA Barcode Blockers - 48 for SeqCap
514134	NEXTflex™ DNA Barcode Blockers - 96 for SeqCap

5142-01	NEXTflex™ PCR-Free DNA Sequencing Kit (8 reactions)
5142-02	NEXTflex™ PCR-Free DNA Sequencing Kit (48 reactions)
514110	NEXTflex™ PCR-Free Barcodes – 6
514111	NEXTflex™ PCR-Free Barcodes – 12
514112	NEXTflex™ PCR-Free Barcodes – 24
514113	NEXTflex™ PCR-Free Barcodes – 48

DNA Fragmentation

Catalog #	Product
5135-01	AIR™ DNA Fragmentation Kit (10 reactions)
5135-02	AIR™ DNA Fragmentation Kit (40 reactions)

WE WANT TO HEAR FROM YOU!

Your feedback is important to us. Tell us what you think of our kits by scanning the QR code or visiting our website at www.biooscientific.com/NGSfeedback. We can't wait to hear from you!